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Flagellar Motility Confers Epiphytic Fitness Advantages upon *Pseudomonas syringae*

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The role of flagellar motility in determining the epiphytic fitness of an ice-nucleation-active strain of *Pseudomonas syringae* was examined. The loss of flagellar motility reduced the epiphytic fitness of a normally motile *P. syringae* strain as measured by its growth, survival, and competitive ability on bean leaf surfaces. Equal population sizes of motile parental or nonmotile mutant *P. syringae* strains were maintained on bean plants for at least 5 days following the inoculation of fully expanded primary leaves. However, when bean seedlings were inoculated before the primary leaves had expanded and bacterial populations on these leaves were quantified at full expansion, the population size of the nonmotile derivative strain reached only 0.9% that of either the motile parental or revertant strain. When fully expanded bean primary leaves were coinoculated with equal numbers of motile and nonmotile cells, the population size of a nonmotile derivative strain was one-third of that of the motile parental or revertant strain after 8 days. Motile and nonmotile cells were exposed in vitro and on plants to UV radiation and desiccating conditions. The motile and nonmotile strains exhibited equal resistance to both stresses in vitro. However, the population size of a nonmotile strain on leaves was less than 20% that of a motile revertant strain when sampled immediately after UV irradiation. Epiphytic populations of both motile and nonmotile *P. syringae* declined under desiccating conditions on plants, and after 8 days, the population size of a nonmotile strain was less than one-third that of the motile parental or revertant strain.

Flagellar motility allows bacteria to respond to favorable or unfavorable stimuli in their environment, thereby increasing the probability of survival (7, 10, 25, 29). In the phylloplane, the ability to respond with motion to stimuli (i.e., chemotaxis) may facilitate survival or avoidance of environmental stress (e.g., competition for habitat and nutrients and survival under desiccating conditions or of UV irradiation) (12, 28). The chemotactic and motility responses of the epiphytic phytopathogen *Erwinia amylovora* were shown to be influenced by temperature, pH, organic compounds, and chelating agents (26, 27). *Pseudomonas syringae* pv. *lachrymans*, another plant pathogen, is attracted by plant extracts and to water droplets collected from leaf surfaces (3). Leben and co-workers (11, 13) reported that the macromovement or migration of bacteria on plant surfaces is assisted by motility as well as by gravitational and capillary forces and requires high relative humidity. Bacterial adherence to plant surfaces has been reported to be independent of motility (15). Motility has also been reported to increase the infectivity of *P. syringae* pv. *phaseolicola* (25). No studies have been made of the influence of motility on survival or of the interaction of bacteria on leaf surfaces. Such studies should better define the epiphytic habitat of bacteria on plants. Ice-nucleation-active (INA) bacteria are ideal organisms with which to study these subtle changes in in situ epiphytic fitness. The probability of plant freezing injury is related to the logarithm of the population size of INA bacteria (17, 18), since most plants produce no ice nuclei active above −5°C. The frequency with which a cell within a population of cells of an INA species produces ice nuclei active at warm temperatures is easily quantified and is a reflection of the conditions under which the cells are grown (18). Because ice nucleus production influences the ability of a leaf colonized by INA bacteria to supercool, i.e., to cool below 0°C without freezing, it is also a measure of the number and conditions of growth of INA bacteria on the leaf surface. Plants can be grown in the greenhouse that, while not axenic, avoid significant populations of INA bacteria and thus are amenable for studies of the behavior of exogenously applied INA bacterial strains. This paper reports findings of the influence of flagellar motility on the epiphytic fitness of an INA strain of *P. syringae*.

MATERIALS AND METHODS

Bacterial cultures. Some characteristics of strain 31, an epiphytic INA bacterium originally isolated by S. E. Lindow, have been previously reported (2). On the basis of its response to standard physiological tests, strain 31 was identified as *P. syringae*. Strain 31R1 is a spontaneous mutant of strain 31 resistant to 100 μg of rifampin per ml. Strain 31R1 was not pathogenic to bean plants or to any of 18 other plant species tested by injecting ca. 10^6 cells into stems and leaves and incubating them for 10 days under moist conditions suitable for disease development.

Mutant strains were produced by treating cells of strain 31R1 grown to the logarithmic growth phase in King medium B (KB; 9) broth with 5% (vol/vol) methanesulfonic acid–ethyl ester for 20 min. Nonmotile mutant strain 3Mot$^-$ and motile revertant strain 3Mot$^+$ were isolated by using the techniques of Panopoulos and Schroth (25). Motile revertants appeared in 1 to 4 days as flares of bacteria spreading away from the center of a stab-inoculated petri plate containing semisolid tryptone medium (23). Light microscopy was also used to detect active bacterial movement (1). The frequency of such events was estimated to be 10^{-9} to 10^{-10}

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per cell, which was similar to the frequency of reversion reported by Panopoulos and Schroth for *P. syringae* pv. *phaseolicola* (25). All strains were resistant to 100 μg of rifampin per ml. A derivative of 3Mot$^-$ was additionally selected for resistance to 100 μg of streptomycin per ml for use in coinoculation experiments. Motility assays were conducted before and during all experiments to confirm this phenotype. Stocks were maintained at 4°C on slants containing nutrient agar amended with 2.5% (vol/vol) glycerol.

The 60 bacterial antagonists surveyed for motility were from the collection of S. E. Lindow (19). These isolates were selected as the predominant bacterial strains from the surfaces of healthy green plants and for the ability to reduce subsequent colonization of plants by INA bacteria and concomitantly reduce frost injury when the host plants were exposed to temperatures between −3 and −5°C. The activities and properties of some of these strains have been reported previously (17, 19, 21, 22).

Plant material. Common bean (*Phaseolus vulgaris* L. var. Bountiful) was grown in UC soil mix (24) in 7.6-cm-diameter clay pots. The leaves of plants were kept dry during propagation to avoid large contaminating epiphytic bacterial populations, including INA bacteria. No bacterial ice nuclei or INA bacteria were detected on uninoculated plants. The total number of bacteria recovered on bean plants was always less than 5×10^4 cells per g (fresh weight). All experiments involving plants were carried out in a greenhouse maintained at 23 ± 3°C.

Field surveys. Leaf samples were collected from Tulare County, Calif., in the spring of 1983. Dry leaves of tomato (*Lycopersicon esculentum* Mill.), navel orange (*Citrus sinensis* Osbeck), wheat (*Triticum aestivum* L.), olive (*Olea europaea* L.), onion (*Allium cepa* L.), and grape (*Vitis vinifera* L.) were sampled in the afternoon and were transported to Berkeley in sterile plastic bags inside a styrofoam cooler held at 5°C. Samples were processed within 48 h of collection.

Quantification of bacterial populations. Populations of bacteria on leaf surfaces were quantified by dilution plating of leaf washings. Individual leaves were weighed and submerged in flasks containing 0.1 M potassium phosphate buffer (pH 7.0) supplemented with 0.1% (wt/vol) peptone, which were shaken vigorously by hand for 30 s and placed in an ultrasonic bath (Bransonic model 72) for 10 min at 0°C (D. M. Haefele and R. R. Webb, Phytopathology **72**:947, 1982). Bacteria released into the washing buffer were quantified by dilution plating onto KB amended as appropriate with rifampin, streptomycin, or cycloheximide each at 100 μg/ml or benomyl (Benlate; Du Pont Co.) or dichloran (Botran; The Upjohn Co.) each at 50 μg/ml. When plants were coinoculated with two or more strains, the strains were differentiated by growh on two or more appropriate antibiotic-containing media. At least 10 individual leaves were assayed for each treatment unless otherwise indicated.

Quantification of warm-temperature ice nuclei. A droplet-freezing assay of leaf washings or bacterial suspensions was used to quantify warm-temperature ice nuclei (20). The mean supercooling point, i.e., the mean temperature to which individual leaves could supercool and thus avoid freezing, was also determined by a method similar to that of Hirano et al. (5). A total of 40 individual bean leaves per treatment were collected, and each was immersed in a test tube containing 10 ml of water free of ice nuclei at temperatures above −6°C. All tubes were cooled in a circulating refrigerated ethanol bath at −1.5°C for 30 min. The ethanol was circulated vigorously, and the temperature was maintained at ±0.05°C. The bath temperature was then lowered by 0.5°C at each 20-min interval until the bath temperature reached −6°C. All tubes reached temperature equilibrium with the circulating coolant in this time interval. The cumulative number of tubes frozen at each temperature interval of 0.5°C was recorded. Each leaf that froze within a temperature interval was assigned a freezing temperature equal to the lower temperature of that interval. Although the distribution of tube freezing temperatures approached a normal distribution for most samples, the distribution was not normal for all samples. However, the central limit theorem allows the use of normal statistics for evaluating the mean supercooling point when the number of estimates of the mean is sufficiently large (16). Because we examined 40 samples of bean leaves (except when noted), we feel that this criterion has been met, and we have used Student's *t* tests (least significant difference) to compare the mean supercooling points of these leaves.

Plant inoculation. Bean plants were inoculated with parental and mutant strains either singly or in mixtures. Inocula were prepared from 48-h cultures grown on KB. Approximately 1 ml of an aqueous suspension containing 5×10^7 cells per ml (unless otherwise indicated) was sprayed per plant onto fully expanded primary leaves by using a hand-held atomizing sprayer. The plants were placed immediately in a closed plastic tent on a greenhouse bench at 21°C, where they were continuously misted with distilled water from four compressed-air-driven atomizers. Bean seedlings were inoculated with a droplet of a cell suspension as the hypocotyl broke the soil surface, placed in the mist chamber, and sampled when the primary leaves were fully expanded.

UV irradiation. Cells were grown in KB broth for 24 h, harvested by centrifugation, washed twice, and suspended in sterile 0.1 M $MgSO_4$ to a final cell concentration of 2×10^9 cells per ml. A cell suspension containing 5×10^5 cells per ml was obtained by dilution of the stock culture with sterile distilled water and subjected to 0, 20, 40, and 80 J of UV radiation (λ254 nm) per ml in open, glass petri dishes in the dark. The number of surviving cells was estimated after each exposure by dilution plating onto KB conducted under red light. Spray-inoculated plants were left on a dry greenhouse bench for 18 h, leaf samples were collected and irradiated, and the remaining plants were placed in a mist tent. After 90 h, the plants were removed from the mist tent and allowed to air dry on open greenhouse benches until all surface water disappeared (ca. 1 h). The population size of each strain was determined from 10 individual dry-irradiated (62 J/m^2 on each side) and 10 nonirradiated leaves by dilution plating of leaf washings conducted in red light after each of the two incubation periods. Population sizes were log transformed, and analysis of variance and mean comparisons were performed by using the General Linear Models procedure provided by Statistical Analysis Systems (SAS release 5.15; SAS Institute Inc., Cary, N.C.).

In vitro desiccation survival assays. For each isolate, two different tests were performed. A 10-ml sample from a culture grown in KB broth for 24 h was placed in an open, plastic petri dish in a sterile laminar flow hood and dried for 48 h. The initial cell concentration was determined by dilution plating. At 48 h after drying, the dried material in each dish was suspended by vigorous washing in 2 ml of sterile distilled water. The number of surviving cells was quantified by dilution plating of these washings. A known number of cells from the same source was also lyophilized to compare survival characteristics under different desiccating conditions. Sterile distilled water (2 ml) was added to the

TABLE 1. Population sizes of three *P. syringae* strains on bean primary leaves and the mean supercooling point of these leaves 5 days after inoculation of seedling hypocotyls with equal numbers of either strain

Strain	No. of bacteria recovered {log [CFU/g (fresh wt)]}[a]	Mean supercooling point (°C)[a]
31R1	3.39b	−2.5b
3Mot$^-$	1.34c	−2.8c
3Mot$^+$	3.37b	−2.6b

[a] Means within a column that are followed by the same letter (b or c) do not differ significantly ($P = 0.05$; $n = 18$) by Duncan's multiple range test.

lyophilized cells, and the number of surviving cells was determined by dilution plating on KB.

RESULTS

Nonmotile mutants of strain 31R1 were easily identified after mutagenesis as described above. Nonmotile mutants were obtained at a frequency of ca. 3×10^{-4} per cell surviving mutagenesis. The nonmotile mutant 3Mot$^-$ was identical to wild-type strain 31R1 in all characteristics measured except the expression of ice nuclei active at −5°C. This isolate also reverted to a fully motile phenotype at a frequency of ca. 1×10^{-10} per cell. One such revertant strain, 3Mot$^+$, was identical in all characteristics measured (except motility) to strain 3Mot$^-$. In replicated trials, the rate of spread in semisolid tryptone medium of 3Mot$^-$ (0.03 mm/h) differed significantly ($P = 0.05$) from those of 31R1 and 3Mot$^+$ (0.55 and 0.51 mm/h, respectively). The rates of spread of 31R1 and 3Mot$^+$ did not differ significantly. All strains were prototrophic, and generation times in KB from replicated trials for 31R1, 3Mot$^-$, and 3Mot$^+$ (91.2, 96.1, and 96.5 min, respectively) did not differ significantly ($P = 0.05$). When assayed at −9°C, no significant differences were seen in nucleation frequency between any of the strains (31R1, 3Mot$^-$, and 3Mot$^+$ had ice nucleation frequencies of 6.6, 7.1, and 4.9 cells per ice nucleus, respectively).

When equal cell numbers of strains 31R1, 3Mot$^-$, or 3Mot$^+$ were inoculated onto separate sets of expanded moist bean primary leaves, population sizes measured 3 days after inoculation did not differ significantly ($P = 0.05$) and were 4.94, 5.13, and 5.19, respectively [log(CFU/gram of tissue)]. However, the motile wild-type and revertant strains both produced more ice nuclei on leaves than the nonmotile mutant did. Ice nucleation frequencies of 31R1, 3Mot$^-$, and 3Mot$^+$ were 3.32, 3.04, and 3.56 log cells per ice nucleus, respectively, at −5°C and 3.55, 3.24, and 3.81 log cells per ice nucleus, respectively, at −9°C. Leaves colonized by the two motile strains also supercooled significantly less ($P = 0.05$) than did leaves inoculated with the nonmotile mutant (Table 1). Mean supercooling points for 31R1, 3Mot$^-$, and 3Mot$^+$, respectively, were −2.3, −2.5, and −2.3°C.

The population sizes of motile and nonmotile *P. syringae* strains inoculated singly onto moist bean leaves under slightly different incubation conditions from those described above were similar for the first 5 days after inoculation but diverged later (Fig. 1A). While the motile and nonmotile population sizes were quite similar overall, motile populations increased and nonmotile populations decreased between days 5 and 8 after inoculation. The population sizes of wild-type and revertant motile strains increased by factors of 6.3 and 5.8, respectively, over the course of this study. The population sizes of nonmotile bacteria were similar to those of motile strains until day 5. At day 8, however, only 1.4% of this population remained. The numbers of ice nuclei active at −5 and −9°C on leaves, although declining initially in all treatments, were similar for both motile and nonmotile strains (Fig. 1B and C).

The population sizes of motile and nonmotile *P. syringae* strains on bean primary leaves differed significantly when only hypocotyls were inoculated as plants were just breaking the soil surface (Table 1). The population size of the nonmotile strain was approximately 100-fold lower than the population size of either the motile parental or revertant strain 5 days after inoculation with equivalent inoculum concentrations. The mean supercooling point of leaves colonized by the nonmotile strain was also significantly lower than that of leaves colonized by the motile parental or revertant strain (Table 1).

Bean primary leaves were coinoculated with equal num-

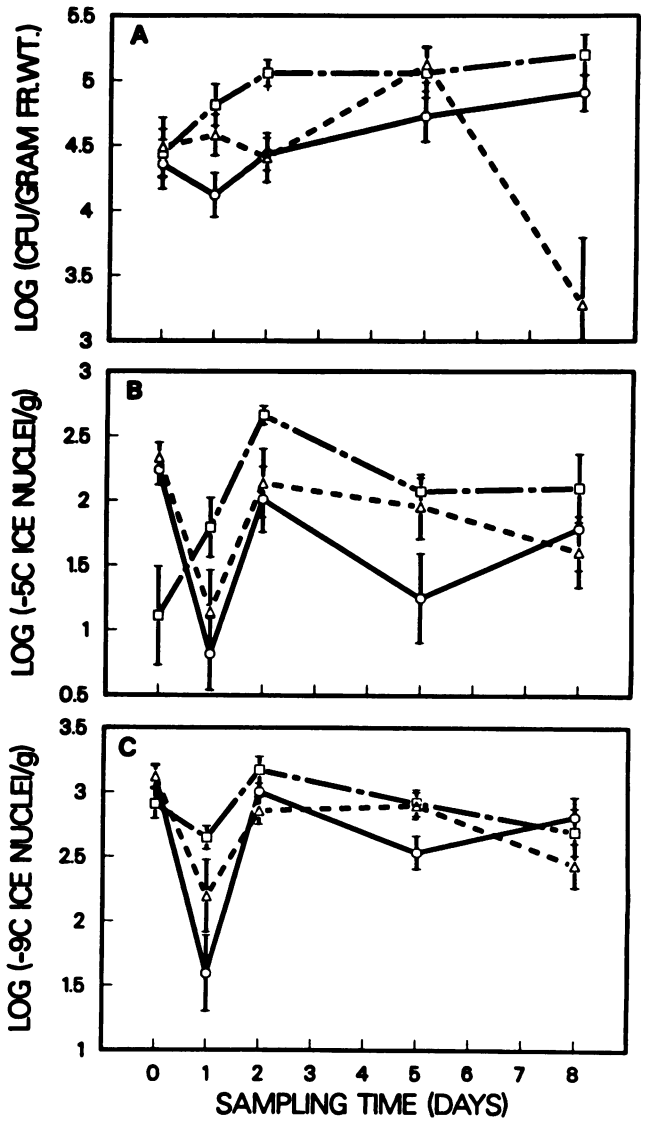

FIG. 1. Population sizes (A), ice nuclei active at −5°C (B), and ice nuclei active at −9°C (C) on bean primary leaves inoculated with *P. syringae* 31R1 (○), 3Mot$^-$ (△), or 3Mot$^+$ (□). The vertical bars represent the standard error of the mean of log CFU or ice nuclei per gram (fresh weight) of tissue.

TABLE 2. Population sizes of three *P. syringae* strains after coinoculation of moist bean leaves with equal numbers of cells of motile and nonmotile strains

Strains tested in combination (motile + nonmotile)[a]	Strain	Bacteria recovered[b] {log[CFU/g (fresh wt)]}	% of total *P. syringae* cells on leaf
31R1 + 3Mot$^-$	31R1	4.45c	76
	3Mot$^-$	3.94d	24
3Mot$^+$ + 3Mot$^-$	3Mot$^+$	4.52c	80
	3Mot$^-$	3.92d	20

[a] Initial inoculum concentration of each strain was 8.5×10^6 CFU/ml. Approximately 1.0 ml of inoculum of each strain was applied per leaf.
[b] Means followed by the same letter (c or d) do not differ significantly ($P = 0.05$; $n = 20$) by Duncan's multiple range test.

bers of cells of motile and nonmotile strains to determine the relative competitive abilities of these strains on leaves. The population sizes of parental and motile revertant strains did not differ, but both were significantly larger than that of the nonmotile mutant strain when quantified after 2 days (Table 2). In another experiment, the population sizes of motile and nonmotile strains were similar for the first 2 days after coinoculation of bean plants (Fig. 2A and B). Subsequently, however, the population sizes of the motile strains 31R1 and 3Mot$^+$ increased, while the number of nonmotile bacteria decreased or increased only slightly depending on the strain mixture (Fig. 2A and B). Significantly fewer nonmotile cells than motile cells were present on leaves 4 and 8 days after inoculation with both mixtures.

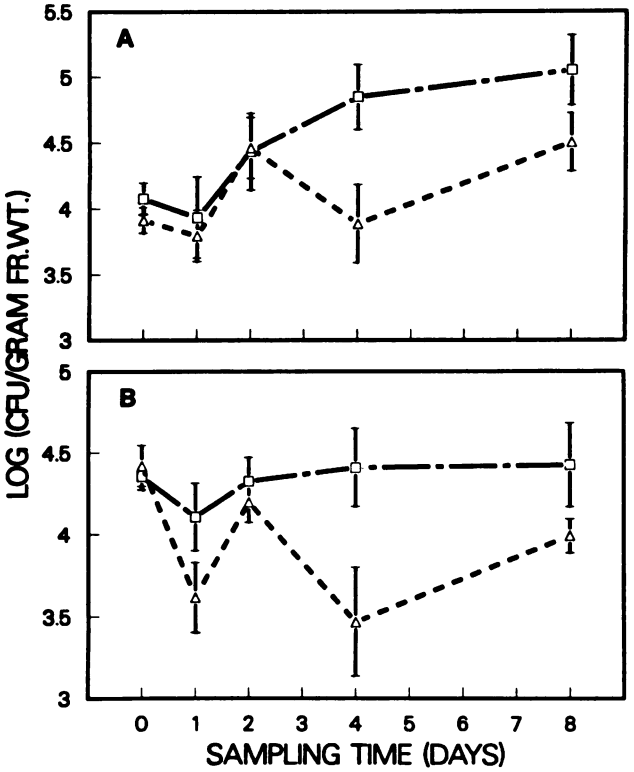

FIG. 2. Population sizes of *P. syringae* 3Mot$^-$ (△) and 3Mot$^+$ (□) (A) or 31R1 (□) and 3Mot$^-$ (△) (B) coinoculated onto bean primary leaves in equal numbers. Vertical bars represent the standard error of the mean of log CFU per gram (fresh weight) of tissue.

TABLE 3. Survival of three *P. syringae* strains on bean leaves irradiated after dry incubation for 18 h after inoculation or irradiated after plants were incubated an additional 72 h in a mist chamber

Moist incubation period (h)	Strain	No. of bacteria recovered with or without UV irradiation {log[CFU/g (fresh wt)]}[a]		% Cells killed
		None	62 J/m^2	
0	31R1	4.61b	3.67b	88.6
	3Mot$^-$	4.68b	3.06c	97.6
	3Mot$^+$	4.70b	3.81b	87.2
72	31R1	4.79b	4.18b	75.5
	3Mot$^-$	3.96c	2.99c	89.3
	3Mot$^+$	4.81b	4.27b	71.0

[a] Means within a column followed by the same letter (b or c) do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

A nonmotile mutant of *P. syringae* 31R1 was more readily killed by UV radiation on leaves than was the motile parental or revertant strain, even though in vitro UV sensitivity did not differ among the strains. The rate of cell death with increasing UV dose of all three isolates in vitro followed first-order kinetics with similar 50% lethal doses. The 50% lethal doses were 12.5, 10.0, and 13.3 J/m^2 for strains 31R1, 3Mot$^-$, and 3Mot$^+$, respectively. While the population sizes of motile and nonmotile strains on bean leaves 18 h after inoculation did not differ prior to irradiation, the number of cells of strain 3Mot$^-$ that survived irradiation was significantly less than that of motile strains (Table 3). The fraction of the population of each strain killed when irradiated after an additional 72 h of moist incubation on leaves was lower than after dry incubation. However, the nonmotile strain again survived irradiation significantly less well than did either the motile parent or revertant strain (Table 3).

Nonmotile cells survived desiccation significantly less well on leaves than did near-isogenic motile strains (Fig. 3). The population sizes of all three strains declined on plant surfaces when the surfaces were allowed to dry for 8 days. However, the population size of the nonmotile strain, although initially significantly higher, was significantly lower after 8 days on dry leaves than that of either the motile parental or revertant strain. The sizes of the two motile populations did not differ at any time under desiccating conditions on leaves. The motile and nonmotile strains of *P. syringae* did not differ significantly in survival of desiccation in vitro. Approximately 2.8, 4.9, and 1.4% of the cells of *P. syringae* 31R1, 3Mot$^-$, and 3Mot$^+$, respectively, survived air drying. Approximately 0.29, 0.28, and 0.31% of these same strains, respectively, survived lyophilization.

Of 1,088 bacterial strains isolated from the surface of six host plants, 76% were motile. Of the bacteria isolated from tomato, 75% of the isolates were motile, while 64, 90, 70, 96, and 61% of the isolates from citrus, wheat, olive, onion, and grape, respectively, were motile. When bacteria antagonistic to INA bacteria on leaf surfaces were assayed for motility, 58 of the 60 strains examined were found to be motile.

DISCUSSION

Approximately 75% of the bacterial isolates from six agricultural plants investigated were motile. Yoshimura (31) found that 75% of the phylloplane bacteria tested from a pine forest were motile. These results substantiate the predom-

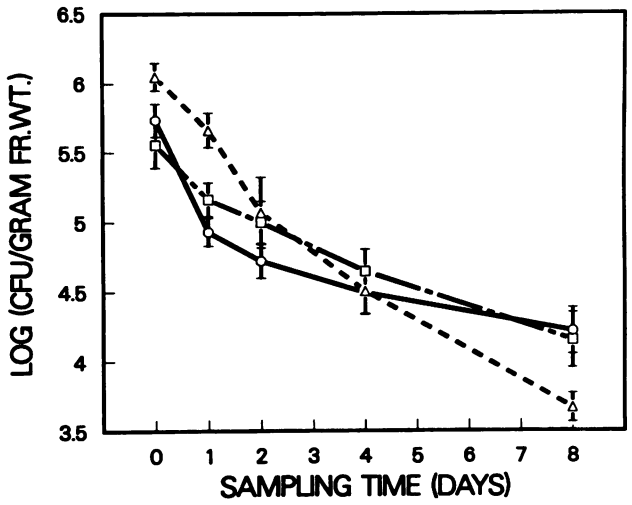

FIG. 3. Population sizes of *P. syringae* 31R1 (○), 3Mot$^-$ (△), and 3Mot$^+$ (□) on bean primary leaves incubated under dry conditions. Bean plants were removed from a mist tent 48 h after inoculation with ca. 5×10^7 cells per ml and placed on a greenhouse bench. The bacterial populations of 10 individual leaves from each treatment were determined immediately and after the times shown on the abscissa. The vertical bars represent the standard error of the mean of log CFU per gram (fresh weight) of tissue.

inance of motile strains among phylloplane bacteria isolated from agricultural ecosystems. The number of motile strains among effective antagonists of INA bacteria on leaf surfaces was much higher (97% motile) than in the population of phylloplane bacteria as a whole. Motility may therefore be a requirement for inter- or intraspecies interactions on leaf surfaces. Since competition for limiting environmental resources, and not antibiosis or other mechanisms, apparently is the basis of antagonism on leaf surfaces (19), the ability to explore the leaf surface may maximize such competition. If motility is critically important in survival on leaf surfaces, it is possible that many nonmotile bacteria detected on leaves have only a transient existence there and originated in habitats where motility is not as necessary for survival.

The loss of motility decreased the ability of a normally motile strain of *P. syringae* to establish a large stable population on bean leaf surfaces, to produce ice nuclei, to colonize new expanding bean tissue, to compete on leaves, and to survive the stresses of desiccation and exposure to UV radiation on leaf surfaces. The possibility that the nonmotile mutant has phenotypic defects independent of motility but due to the mutation of a gene conferring motility cannot easily be discounted. Such pleiotropic effects were not evident in strain 3Mot$^-$ in vitro. Leben and co-workers (13, 14) found that motile epiphytes are able to colonize terminal buds and newly unfurled leaves when buds alone are inoculated, but due to the lack of available isogenic nonmotile strains, they were unable to attribute this ability to motility. While some colonization of distal plant parts occurred in our experiments, presumably by passive movement such as bulk water flow, the number of nonmotile strains observed to move to distal leaves was much lower than for motile strains. Even when differences in the population sizes of motile and nonmotile strains were not observed, differences in their production of ice nuclei and of the supercooling capacity of leaves colonized by these strains occurred. Since the production of ice nuclei is strongly controlled by the physiological state of INA bacteria (18), differences in the environments that motile and nonmotile strains encountered or differences in their responses to the environment seem apparent.

Motile and nonmotile *P. syringae* strains exhibited distinctly different responses to physical stresses on leaf surfaces. The higher susceptibility of nonmotile *P. syringae* strains to killing by UV radiation on leaves may represent increased physical exposure of nonmotile strains to the nonpenetrating radiation relative to motile *P. syringae* strains. A portion of the motile bacteria may survive irradiation in sites shaded by surface features, e.g., trichomes, anticlinal wall junctions, or epicuticular wax crystals (30) or within stomata or hydathodes. Motility may aid in their ability to colonize such sites. Kennedy and Ercolani (8) concluded that the higher percent recovery of motile cells of the plant pathogen *P. syringae* pv. *glycinea* from irradiated leaves is the result of the infiltration of the leaf mesophyll by more motile than nonmotile cells. Epiphytic populations of nonpathogenic, presumably commensal, bacteria appear to be more strictly limited to plant surfaces (D. M. Haefele and S. E. Lindow, Phytopathology, **72**:946, 1982). While only viable cells that could be washed off plants by extensive sonication were measured in these studies, other studies have shown that washing removes from 60 to 95% of all bacteria compared with those that are recovered after maceration of tissue (R. D. O'Brien and S. E. Lindow, Phytopathology **76**:1068, 1986). Thus, differential recovery efficiencies of motile and nonmotile strains are unlikely.

The decreased survival of nonmotile *P. syringae* populations under desiccating conditions on plant surfaces may also result from the more exposed physical habitats to which nonmotile cells may be restricted on leaf surfaces. Gross leaf surface morphology has been shown to influence bacterial survival of desiccation on plant surfaces (4). Bacterial survival of desiccation is higher on pubescent leaves than on glaborous leaves (4). The differences in survival of motile and nonmotile *P. syringae* on bean leaves having presumably one morphotype may reflect the importance of microsite characteristics for survival and the requirement that cells reach such sites for survival. Variations of bacterial population size between leaves and within different portions of the same leaf (6) may reflect such a distribution of microsites among leaves and on a given leaf.

There may be areas of the normal leaf surface with one or more characteristics which motile, but not nonmotile, epiphytic INA *P. syringae* exploit and thereby increase their probability of survival. The lower probability of survival of the nonmotile mutant of *P. syringae* on the phylloplane demonstrates directly the importance of motility and, by inference, the relative desirability of habitat which motile bacteria are likely to encounter. A better understanding of the habitat requirements of epiphytic bacteria may allow the selection of bacterial strains or species with survival requirements complementary to those provided by a given plant species. Conversely, it may be possible to alter the physical or chemical characteristics of leaf surfaces by plant breeding to reduce their ability to support populations of certain deleterious epiphytic bacteria.

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